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Distribution of Sequences common to the 25–28S-Ribonucleic Acid Genes of *Xenopus laevis* and *Neurospora crassa*

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The extent of homology between the nucleotide sequence of L-rRNA (the major RNA component of the larger ribosomal subparticle) of a lower eukaryote (*Neurospora crassa*) and an amphibian (*Xenopus laevis*) was investigated by utilizing rDNA (DNA coding for rRNA) of *X. laevis* cloned in plasmids pMB9 and pML2, and rDNA of *N. crassa* cloned in bacteriophage λ. Hybridization studies revealed that sequences common to both *N. crassa* and *X. laevis* L-rRNA comprise a total of approx. 1050 ± 200 nucleotides. The thermal stability of the *X. laevis* rDNA·*N. crassa* L-rRNA hybrid was 5°C lower than that of the *X. laevis* rDNA·*X. laevis* L-rRNA duplex, indicating the presence of fewer than 10% mismatches in homologous sequences. *X. laevis* rDNA was analysed by means of restriction endonucleases and hybridization with ^{125}I-labelled *N. crassa* L-rRNA. Most (at least 95%) of the conserved sequences were present in a 3000-base-pair fragment produced by restriction with endonucleases *Hin*dIII and *Bam*HI. This fragment, which includes the 3′-OH terminus of the L-rRNA-coding region, was used as an adaptor in the construction of a bacteriophage-λ recombinant. One section of the recombinant phage terminating in a *Hin*dIII-specific site was obtained from bacteriophage λ p*lac*5 (after restriction with endonuclease *Hin*dIII). A second section terminating in a *Bam*HI-specific site was obtained from bacteriophage λ 540 (after restriction with endonuclease *Bam*HI). These two parts were joined by means of the *X. laevis* rDNA fragment. Further analysis of cloned rDNA by means of restriction endonucleases confirmed that conserved sequences were widely distributed throughout the 3000-base-pair fragment produced by *Hin*dIII and *Bam*HI endonucleases. A 3400-base-pair fragment of *N. crassa* rDNA cloned in a bacteriophage λ [Cox & Peden (1979) *Mol. Gen. Genet.* **174**, 17–24] was restricted with endonucleases. The products were hybridized with ^{125}I-labelled *X. laevis* L-rRNA. Conserved sequences were shown to be distributed over a range of approx. 1600–2700 base-pairs. Hence, in neither *X. laevis* nor *N. crassa* L-rRNA can the conserved sequences form a single block; instead regions of high and low (or no) homology must be intermingled. Both *N. crassa* rDNA and *X. laevis* rDNA were found to hybridize with *Drosophila melanogaster* L-rRNA sequences. Those rDNA fragments with sequences common to *X. laevis* and *N. crassa* L-rRNA also hybridized with *D. melanogaster* L-rRNA probe. Thus the same set of conserved sequences may be present in all three species.

Abbreviations used: S-rRNA and L-rRNA are the major RNA species of the smaller and larger ribosomal subparticles respectively. s*Hin*dIII 5.2 etc. indicates a site for endonuclease *Hin*dIII etc. located at 5.2 kbp etc. from the reference point. s*Eco*RI 3120 base-pairs to s*Eco*RI 3710 base-pairs etc. indicates a fragment obtained by cleavage at the sites indicated. SDS, sodium dodecyl sulphate; DNAase, deoxyribonuclease; RNAase, ribonuclease; SSC, 0.15 M-NaCl/0.015 M-sodium citrate, pH 7.0; T_m, the temperature at which 50% of the duplex was dissociated into single strands, as measured by the release of the radioactive strand into solution; kbp, kilo-base-pairs, a measure of the size of DNA.

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Studies of genes for cytoplasmic rRNA have proved to be informative about gene organization and gene expression, including special mechanisms for gene amplification. The rRNA genes are organized in clusters, and the genes within the cluster appear to have evolved in parallel. Studies of *Xenopus laevis* rRNA have been made generally with these problems in mind (for review see Wellauer & Dawid, 1977).

Although rRNA genes have evolved, as may be seen from the increase in the size of the L-rRNA component and in the changes of nucleotide composition (Lava-Sanchez *et al.*, 1972; Loening, 1968), there are elements that are strongly conserved, as shown by hybridization studies (Martin *et al.*, 1970; Sinclair & Brown, 1971; Birnstiel & Grunstein, 1972; Gerbi, 1976). These trends agree with the notion that S-rRNA and L-rRNA include a conserved core and that tracts of non-conserved sequences account for the variations in mass and nucleotide composition (for review see Cox, 1977). The conserved sequences are of interest because they are probably important to ribosome function.

The aim of this work was to establish the extent of homology between the nucleotide sequences of L-rRNA of a lower eukaryote (*Neurospora crassa*) and an amphibian (*X. laevis*) and to identify the location of conserved sequences. The existence of homologies between *N. crassa* and *X. laevis* L-rRNA was utilized when a *X. laevis* rDNA (DNA coding for rRNA) probe was used to identify *N. crassa* rDNA cloned in bacteriophage λ (Cox & Peden, 1978, 1979). The study of conserved parts of L-rRNA is more conveniently approached through the rRNA genes because of the wide range of available endonucleases that cleave at specific sites. For this reason *X. laevis* rDNA cloned in plasmids and *N. crassa* rDNA cloned in bacteriophage λ were used in the present study. Restriction endonucleases were used to cleave rDNA of both *X. laevis* and *N. crassa*, and the fragments were separated and examined for the presence of conserved sequences by measuring their capacity to hybridize with radioactively labelled heterologous L-rRNA by using the technique of Southern (1975) transfers.

To test the view that the sequences common to *X. laevis* and *N. crassa* represent a feature of cytoplasmic L-rRNA in general, the capacity of *X. laevis* and *N. crassa* rDNA to hybridize with *Drosophila melanogaster* L-rRNA sequences was also investigated.

It was briefly reported (Cox & Thompson, 1978) that the conserved sequences of the *X. laevis* L-rRNA were located in a 3000-base-pairs fragment that stretches from the 3'-OH terminus. This rDNA fragment, which was produced by restriction of rDNA with endonucleases *Hin*dIII and *Bam*HI, was shown to hybridize with both ^{125}I-labelled *N. crassa* L-rRNA and with nick-translated *D. melanogaster* rDNA.

Experimental

Materials

Enzymes. Bacteriophage-T_4 polynucleotide ligase was brought from Miles Laboratories, Elkhart, IN, U.S.A. Restriction enzymes were made available by Dr. K. Murray, Department of Molecular Biology, University of Edinburgh. Enzyme *Eco*RI was also purchased from the Microbiological Research Establishment, Porton, Wiltshire, U.K., and *Bam*HI was also bought from New England Bio-Labs, Beverley, MA, U.S.A. Plasmid pX1r101 (constructed by Dr. R. Reeder) was given by Dr. A. Bird, Department of Zoology, University of Edinburgh; plasmid p4305 was constructed and given by Dr. J. O. Bishop, Department of Animal Genetics, University of Edinburgh; plasmids pDm103, CKDm103A and CKDm103C (Glover & Hogness, 1977) were given by Dr. D. Glover, Department of Biochemistry, Imperial College, University of London.

Radioisotopes. Carrier-free [^{125}I]iodide (100mCi/ml) and deoxyribonucleoside [α-^{32}P]triphosphates were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Nitrocellulose film was bought from Schleicher and Schull, Dassel, Federal Republic of Germany.

Preparation of plasmid DNA

Plasmid DNA was purified from bacterial lysates by banding twice in CsCl/ethidium bromide gradients (Clewell & Helinski, 1969). Ethidium bromide was removed by dialysis against 200mM-NaCl/10mM-Tris/HCl (pH 8.0)/1mM-EDTA at 4°C for 24h.

Isolation of bacteriophage-λ DNA

Bacteriophage λ with inserted *N. crassa* rDNA or *X. laevis* rDNA was grown in liquid culture, isolated by differential centrifuging and purified by banding in CsCl density gradients (Murray *et al.*, 1977). Purified bacteriophage were dialysed against 1mM-EDTA/0.01M-Tris/HCl, pH7.6 to remove CsCl; DNA was then isolated by extraction with phenol, dialysed against 1mM-EDTA/0.01M-Tris/HCl, pH7.0, and stored at 0°C.

^{32}P labelling of DNA in vitro by nick translation

The protocol of Maniatis *et al.* (1975) was followed, except that the unlabelled deoxyribonucleoside triphosphate concentration was 10μM each and the DNAase I concentration was between 10 and 50ng/ml depending on the activity of the enzyme, which was assayed beforehand. Reaction volume was 100μl and usually 0.5–1μg of DNA was labelled. After nick translation the reaction

mixture was extracted twice with phenol, once with chloroform/octan-2-ol (12:1, v/v) and once with diethyl ether, and fractionated on a Sephadex G-50 column (10cm long × 1cm diameter) in 10mM-NaCl/10mM-Tris/HCl (pH 8.0)/1mM-EDTA/0.2% SDS. Radioactivity was monitored by using Cerenkov radiation and the excluded material was pooled. Specific radioactivities were between 1×10^7 and 5×10^7 c.p.m./μg of DNA.

Restriction of DNA

The buffer used for restriction was generally 10mM-$MgCl_2$ / 10mM-2-mercaptoethanol/50mM-NaCl/10mM-Tris/HCl, pH 7.5, but the concentration of NaCl was 100mM for restriction with endonuclease *Eco*RI. The reactions were carried out in small plastic tubes, fitted with caps, kept at 37°C (except for endonuclease-*Bam*HI digests, which were kept at 30°C) for 1–3h according to the enzymic activity. At the end of the reaction the tubes were placed for 10min in a water bath maintained at 70°C and then plunged into an ice bath.

Gel electrophoresis

Agarose gels (1.0 or 1.5%, w/v) were prepared. The buffer (gel buffer) used for casting and for running the gel was 0.02M-sodium acetate/1mM-EDTA/0.04M-Tris/acetate buffer, pH 8.2. The gels were run at approx. 20°C for 16–18h; the current was 20mA and the voltage was 50V. The gels were stained for 30min with ethidium bromide (1mg/litre of gel buffer), destained by soaking in gel buffer for 30min and then photographed in u.v. light.

Molecular-weight calibration was achieved by adding restriction fragments of bacteriophage-λ DNA of known size to the sample being investigated. The standard solution was prepared by mixing equal amounts of bacteriophage-λ DNA restricted with endonuclease *Hin*dIII with bacteriophage-λ DNA restricted with endonuclease *Eco*RI. After the gel was stained and photographed, the intensities of the bands were measured by microdensitometry.

Microdensitometry

Photographs of gels and radioautographs were scanned by using a Zeineh Soft Laser Scanning Densitometer (T. and J. Crump, Rayleigh, Essex, U.K.)

Hybridization of rDNA after Southern transfer

The method followed was essentially that of Southern (1975). DNA was denatured by alkali, neutralized and transferred to nitrocellulose film, and the film was then baked for 90min in a vacuum oven kept at 80°C. When the probe was ^{32}P-labelled DNA, the filters were also treated by the Denhardt (1966) procedure. The hybridization conditions were generally as follows: solvent, 4 × SSC/0.2% SDS kept at 65°C for 24–36h, or 55% (w/v) formamide/5 × SSC/0.5% SDS, kept at 55°C for approx. 36h (cf. Glover *et al.*, 1975). The filters were washed three times at the same temperature as the hybridization reaction with buffer of the same composition as that used for the hybridization step, and then with 2 × SSC containing 20μg of RNAase/ml at approx. 20°C.

Analysis of radioautographs

Microdensitometer measurements of gels and of radioautographs were utilized in an attempt to obtain a reliable guide to the relative capacities of the restriction fragments to hybridize with radioactive probes.

Quantitative data are more likely to be obtained if the following conditions are met: all of the DNA is transferred from the gel and is all retained on the nitrocellulose filter; the probe is smaller in size than the sequence to which it hybridizes; hybridization proceeds to completion; and the response of the film is proportional to the radioactive dose. The efficacy of DNA transfer was monitored by restaining the gel after transfer. No residual DNA was detected in the gel, except for the largest (24700 base-pairs) fragment. DNA fragments of the size-range generated by endonuclease-*Eco*RI digestion of bacteriophage DNA were retained by nitrocellulose to an extent greater than 95% as judged by independent experiments using ^{32}P-labelled SV40 DNA restricted with *Hin*dIII and *Hae*III nucleases. Conditions were made favourable for complete reaction by using an excess of probe for a long time. At least 50% of the radioactivity remained at the end of the reaction, and this residual probe could be used again. The radioactive probes were small (100–150 base-pairs) compared with the size of the restriction fragments under scrutiny.

The film was pre-flashed, and an intensification screen was used to promote a linear dose–response range (Laskey & Mills, 1977). This was checked by comparing each radioautograph with a film exposed for a different period to establish that the relative intensities of particular bands were unchanged. Lengthy exposures eventually led to a marked increase in the relative absorbance of the bands that were faint after short exposures. Earlier work (Cox & Peden, 1979) supports the view that, for fragment in the size range 1000–7000 base-pairs, the precautions outlined above favour quantitative analysis of the radioautographs.

Construction of a recombinant bacteriophage λ from components of bacteriophage λ NM540, bacteriophage λ plac5 and the conserved 3000-base-pairs X. laevis rDNA fragment

The 3000-base-pair fragment of *X. laevis* rDNA, produced by restriction with endonucleases *Bam*HI and *Hin*dIII, which spans the conserved sequences

of the L-rRNA gene (Cox & Thompson, 1978) was cloned in bacteriophage λ by using bacteriophage λ NM540 (Murray & Murray, 1975) and bacteriophage λ *plac*5 (Allet & Bukhari, 1975). The three components of the recombinant were the left-hand arm of bacteriophage-λ *plac*5 DNA (15.9×10^6 daltons), terminating in a *Hin*dIII-specific site at the 3′-OH end, the right-hand arm of bacteriophage-λ 540 DNA (9.6×10^6 daltons), extending from the *Bam*HI-specific site that comprises the 5′-end to the 3′-OH terminus, and the conserved 3000-base-pairs fragment of *X. laevis* rDNA, which served as an adaptor to unite the right- and left-hand arms of bacteriophage-λ DNA.

Bacteriophage λ NM540 DNA (1μg) was restricted with endonuclease *Hin*dIII; bacteriophage-λ *plac*5 (1μg) was restricted with endonuclease *Bam*HI, and plasmid-pX1r101 DNA (2μg) was restricted with endonucleases *Hin*dIII and *Bam*HI. The restriction enzymes were inactivated by heating at 70°C for 10min. The extent of digestion was checked by transfection and electrophoresis through 1% (w/v) agarose (Murray *et al.*, 1977). The plasmid-pX1r101 DNA was incompletely restricted by endonuclease *Bam*HI.

The DNA samples were mixed and incubated with bacteriophage-T_4 polynucleotide ligase, as previously described (Murray & Murray, 1974). Bacteriophages were recovered by transfection of a *lacZ Escherichia coli* recipient with bacteriophage DNA. The transfectants were recovered on BBL plates (Murray & Murray, 1975) supplemented with 40mg of 5-bromo-4-chloroindol-3-yl β-D-galactoside/litre (Horwitz *et al.*, 1964) to detect $lacZ^+$ plaques. In view of the distribution of restriction targets, it was expected that most $lacZ^+$ bacteriophages recovered would also be imm_{21}; this was confirmed by testing for growth on an imm_{21} lysogen.

Ten $lacZ^+$ imm_{21} phages were plaque-purified and tested by plaque hybridization for the presence of sequences complementary to a ^{125}I-labelled *Neurospora crassa* L-rRNA probe. Three clones (bacteriophage λ RT8, bacteriophage λ RT9 and bacteriophage λ RT10), which reacted with the probe, were propagated and DNA was prepared. The DNA samples were restricted with both *Hin*dIII and *Bam*HI endonucleases in a double digestion. The fragments were separated by electrophoresis in 1% agarose gels, transferred to nitrocellulose film by the Southern (1975) method and hybridized with ^{125}I-labelled *N. crassa* L-rRNA at 65°C in $4 \times$SSC/0.2% SDS for 16h. The 3000-base-pair rDNA fragment was found to be present in each recombinant. Bacteriophage λ RT9 contained an additional rDNA fragment (fragment 5, Fig. 4); bacteriophage-λ RT10 DNA was found to include a fragment of the vector moiety, pMB9, of plasmid pX1r101.

Isolation of X. laevis L-rRNA

Ribosomes were isolated from *X. laevis* oocytes and were dissociated into subparticles, which were then separated by zonal centrifugation, by the methods described by Pratt & Cox (1971). Fractions containing L-subparticles were combined and the subparticles were recovered by centrifugation. RNA was isolated by precipitation as the guanidinium salt (Cox, 1966).

Isolation of N. crassa L-rRNA

Ribosomes were isolated from *N. crassa* (strain 74A) after disruption of the hyphae with acid-washed sand. The subribosomal particles were isolated by the method developed for rabbit subribosomal particles (Cox & Hirst, 1976). rRNA was isolated from the subparticles by precipitation as the guanidinium salt (Cox, 1966).

Iodination of rRNA

A sample (10μl) of [^{125}I]iodide solution (100mCi/ml) was adjusted to pH4.3 by the addition (4μl) of 0.7M-sodium acetate buffer, pH4.3. *N. crassa* rRNA (50μl of a 1μg/μl solution) was added, followed by 8μl of 12mM-thallium trichloride/0.2M-sodium acetate buffer, pH4.6. The reaction mixture was kept at 60°C for 20min. The solution was then adjusted to pH7 by the addition of 1M-sodium phosphate buffer, pH7.0 (100μl), and 0.1M-sodium sulphite (30μl) was also added. The solution was kept at 60°C for 1h, cooled, carrier *Escherichia coli* rRNA (200μg) was added, the solution was dialysed against 0.1M-NaCl/0.01M-Tris/HCl, pH7.0, and RNA was precipitated at 0°C by the addition of 2.5 vol. of ethanol. The RNA was separated by centrifugation and dissolved in 0.1M-NaCl/0.01M-Tris/HCl, pH7.0. The specific radioactivity was approx. 5×10^6 c.p.m./μg of rRNA. Samples were counted for radioactivity with a Packard Auto-Gamma counter (Packard Instrument Co., Downers Grove, IL. U.S.A.).

Hybridization on nitrocellulose discs

The procedure followed was essentially that of Gillespie & Spiegelman (1965). Plasmid DNA was first restricted with *Eco*RI endonuclease to yield a linear product. DNA samples were denatured by the addition of 0.1 vol. of 1M-NaOH and the solution was kept at 37°C for 10min. The solution was then neutralized with 0.1vol. of 3M-sodium acetate buffer, pH5.0. The DNA solution was then trapped on nitrocellulose discs, which were then mixed in $2 \times$SSC. The dried discs were kept *in vacuo* at 80°C for 90min. Up to six discs were placed in a small vial containing $4 \times$SSC/0.1% SDS, and an excess of ^{125}I-labelled L-rRNA probe (5×10^5c.p.m.) was added. The vials were kept at 65°C for 16h, and were then washed three times with $4 \times$SSC/0.1%

SDS (total 400ml) for 2h at 65°C, and finally were mixed with 2 × SSC at 22°C and then dried with blotting paper.

RNAase treatment of RNA hybridized with filter-bound DNA

The procedure described by Gerbi (1976) was followed, except that treatment with a lower amount of RNAase was shown to be sufficient. Filter discs were immersed in RNAase solution (1ml of a solution of 1.3μg of pancreatic RNAase/ml of 2 × SSC) for 45min at 22°C. The discs were rinsed in buffer and were then kept in 0.1% (v/v) diethyl pyrocarbonate in 2 × SSC for 30min at 22°C. The discs were then washed twice with 2 × SSC and twice more with 0.5 × SSC.

Interpretation of hybridization data

The extent of homology, i.e. the mass of *N. crassa* L-rRNA gene hybridizing with *X. laevis* L-rRNA (or the mass of *X. laevis* L-rRNA gene hybridizing with *N. crassa* L-rRNA), was calculated from hybridization data obtained by using an excess of L-rRNA probe. Suppose that C_1 is the radioactivity of *N. crassa* DNA hybridized with *N. crassa* L-rRNA, C_2 is the radioactivity of *N. crassa* DNA hybridized with *X. laevis* L-rRNA, C_3 is the radioactivity of *X. laevis* DNA hybridized with *X. laevis* L-rRNA, and C_4 is the radioactivity of *X. laevis* DNA hybridized with *N. crassa* L-rRNA. C_1 etc. refer to the radioactivity found after RNAase treatment and after allowance for radioactivity bound to a control filter in the absence of DNA. The radioactivity of a control filter without DNA was equivalent to the radioactivity of a filter with bound DNA after it was heated to 99°C in 0.5 × SSC. Then:

$$C_1 = a_{Nc}\, s_{Nc} \quad (1)$$

$$C_2 = f_{Nc}\, a_{Nc}\, s_{X1} \quad (2)$$

$$C_3 = a_{X1}\, s_{X1} \quad (3)$$

$$C_4 = f_{X1}\, a_{X1}\, s_{Nc} \quad (4)$$

$$f_{Nc}\, M_{Nc} = f_{X1}\, M_{X1} \quad (5)$$

where a_{Nc} is the amount (e.g. ng) per filter of *N. crassa* DNA coding for L-rRNA, a_{X1} is the amount per filter of *X. laevis* DNA coding for L-rRNA, s_{Nc} is the specific radioactivity (e.g. c.p.m./ng) of ^{125}I-labelled *N. crassa* L-rRNA, s_{X1} is the specific radioactivity of *X. laevis* L-rRNA, f_{Nc} is the fraction of the *N. crassa* L-rRNA gene hybridizing with *X. laevis* L-rRNA, f_{X1} is the fraction of *X. laevis* L-rRNA gene hybridizing with *N. crassa* L-rRNA, M_{Nc} and M_{X1} respectively are the molecular weights of *N. crassa* and *X. laevis* L-rRNA. Three parameters may be obtained from eqns. (1)–(5), namely f_{Nc} (and f_{X1}), s_{Nc}/s_{X1} (the ratio of the specific radioactivities of the two L-rRNA probes) and a_{Nc}/a_{X1} (the ratio of the amounts of L-rRNA genes loaded on to the filters). Eqns. (6)–(8) were obtained by dividing eqn. (1) by eqn. (2), by dividing eqn. (3) by eqn. (4), by dividing eqn. (2) by eqn. (3) and simplifying. Hence:

$$f_{Nc} = \left(\frac{M_{X1}}{M_{Nc}}\,\frac{C_2\,C_4}{C_1\,C_3}\right)^{\frac{1}{2}} \quad (6)$$

$$\frac{s_{Nc}}{s_{X1}} = f_{Nc}\,\frac{C_1}{C_2} \quad (7)$$

$$\frac{a_{Nc}}{a_{X1}} = \frac{1}{f_{Nc}}\,\frac{C_2}{C_3} \quad (8)$$

'Melting' profiles

A nitrocellulose disc with bound DNA·RNA hybrid was placed in a cuvette inserted into an electrically heated copper block, and 0.5 × SSC (1ml) was added. After 10min at 25°C the liquid was removed and set aside until its radioactivity was measured. A fresh sample of solution was introduced, the temperature of the cuvette was raised by 5°C, and after 10min the liquid was removed and the sequence of operations was repeated until a temperature of 99°C was attained. The radioactivity of the filter disc was measured before and after the heating procedure. The radioactivity lost from the filter on heating was recovered in solution. The residual radioactivity corresponded to the radioactivity of a control filter free of bound DNA.

Containment facilities

The isolation of recombinant plasmids and bacteriophage was carried out under Category 2 conditions as described in the Williams report (1976; Command No. 6600, H.M.S.O., London, U.K.). Recombinant DNA was handled under Category 1 conditions. These procedures were approved by the Departmental Biosafety Committee and by the Genetic Manipulation Advisory Group.

Results

The plasmid pX1r101 carries a 12000-base-pairs fragment of the *X. laevis* rRNA gene cluster; included are the genes for S-rRNA (the RNA component of the smaller ribosomal subparticle), L-rRNA and 5.8S rRNA, and also spacer regions (cf. Wellauer & Dawid, 1976). Nick-translated plasmid-pX1r101 DNA was used as a probe for identifying molecular recombinants of bacteriophage-λ DNA and *N. crassa* rDNA (Cox & Peden, 1979). *N. crassa* rDNA coding for L-rRNA were shown to be present in a 3400-base-pairs fragment produced by digestion with endonuclease *Hin*dIII (Cox & Peden, 1979). Quantitative information about the extent and degree of homology between *X.*

laevis and *N. crassa* rDNA was sought by using the filter hybridization technique.

Hybridization studies

Hybridization of *X. laevis* rDNA (plasmid-pX-1r101 DNA) and of *N. crassa* rDNA (bacteriophage-λ N24 DNA) with ^{125}I-labelled L-rRNA from *X. laevis* and *N. crassa* was studied by the technique of Gillespie & Spiegelman (1965). After hybridization the filters were treated with pancreatic RNAase to remove tails of single-stranded RNA (cf. Gerbi, 1976). The results presented in Table 1 show that approx. 20–30% of the radioactivity was removed by the RNAase treatment. From the radioactivities of the filters after RNAase treatment (see Table 1) it was calculated, by using eqn. (6) (see under 'Interpretation of hybridization data' in the Experimental section) that $29 \pm 6\%$ [$0.38(\pm 0.07) \times 10^6$ daltons] of *N. crassa* L-rRNA hybridized with the *X. laevis* L-rRNA gene. The conserved sequences comprise approx. 1050 ± 200 bases (assuming a mass of 363 for the Na^+ salt of the average nucleotide). The fidelity of base-pairing was estimated to be 90% or better from a comparison of the thermal stabilities of the homologous and heterologous DNA·RNA hybrids (see Table 1 and also Fig. 1).

The 'melting' profiles of both homologous and heterologous DNA·RNA hybrids were measured in $0.5 \times$ SSC (see the Experimental section) before and after RNAase treatment. The RNAase treatment diminished the radioactivity of the hybrid by 20–30%, but had a marginal effect on the 'melting' profile and did not affect T_m, the 'melting' temperature (see Fig. 1). The heterologous hybrids *X. laevis* DNA·*N. crassa* L-rRNA and *N. crassa* DNA·*X. laevis* L-rRNA had similar, if not identical, 'melting' properties; the observed T_m was 73°C (Fig. 1), that is 5°C lower than the values of T_m found for the homologous hybrids (cf. Sinclair & Brown, 1971; Gerbi, 1976). The approximation that a 1% mismatch of base residues lowers T_m by 1°C (Bonner *et al.*, 1973) suggests a content of no more than 5% mismatched residues. The relations established by Thomas & Dancis (1973) suggest 1.6 mismatches per 100 nucleotides and unbroken runs of 164 nucleotide pairs on average. Although these estimates should be regarded as no more than approximations, they serve to show the presence of regions of homology that should be readily identified by sequencing methods.

Distribution of sequences common to X. laevis and N. crassa L-rRNA genes

The distribution of regions of *X. laevis* rDNA homologous with *N. crassa* L-rRNA were sought.

Table 1. *Matrix of properties of hybrids formed between rDNA and ^{125}I-labelled L-rRNA*

The procedure of Gillespie & Spiegelman (1965) was used for hybridization. Filters were kept in $4 \times$ SSC/0.1% (w/v) SDS at 65°C for 16h. The specific radioactivity of the probe was approx. 5×10^6 c.p.m./μg of L-rRNA. An excess of ^{125}I-labelled probe (approx. 5×10^5 c.p.m.) was used for each batch of six filters. Samples were assayed in triplicate and different amounts of DNA (0.1–0.5μg) per filter were used. The radioactivity of each filter was measured after treatment with RNAase (1.3μg/ml for 45min at 20°C; see the Experimental section), and the radioactivity of a control filter devoid of DNA was subtracted. The radioactivity of the control filter was essentially the same as the residual radioactivity of filters with DNA after heating to 99°C in $0.5 \times$ SSC. The specific radioactivity of ^{125}I-labelled *N. crassa* L-rRNA was 82% of that of ^{125}I-labelled *X. laevis* L-rRNA, as calculated by means of eqn. (7) (see the Experimental section). The amount of *X. laevis* DNA coding for L-rRNA loaded per disc, calculated by means of eqn. (8) (see the Experimental section), was 32% of the amount of *N. crassa* DNA coding for L-rRNA. Values in parentheses are the radioactivity of the filter expressed as a percentage of radioactivity before RNAase treatment.

	^{125}I-labelled L-rRNA probe					
	X. laevis			*N. crassa*		
DNA species	Radioactivity (c.p.m./filter) after RNAase treatment	T_m(°C)	$10^{-6} \times$ Mol. wt. of RNA moiety of duplex	Radioactivity (c.p.m./filter) after RNAase treatment	T_m(°C)	$10^{-6} \times$ Mol. wt. of RNA moiety of duplex
X. laevis	9069 (77%)	77	1.54*	1996 (69%)	73†	0.36‡
N. crassa	8114 (75%)	73†	0.36‡	26435 (85%)	78	1.3§

* The molecular weight of *X. laevis* L-rRNA was given by Loening (1968).

† A T_m of 77.5°C was also reported by Gerbi (1976) for the heterologous hybrid *Drosophilia hydei* DNA·*X. laevis* rRNA.

‡ The molecular weight of sequences common to both *N. crassa* and *X. laevis* L-rRNA was calculated by means of eqn. (6) (see the Experimental section).

§ The molecular weight of *N. crassa* L-rRNA was reported by Lava-Sanchez *et al.* (1972).

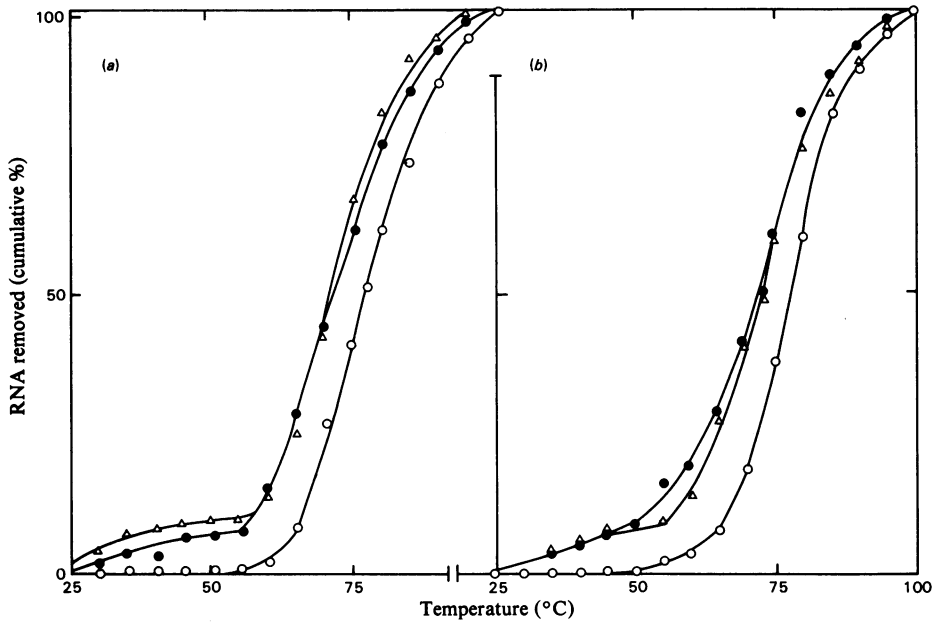

Fig. 1. *'Melting' profiles of rDNA·L-rRNA hybrids*

The 'melting' profile was measured as described in the Experimental section (cf. Gerbi, 1976). The solvent was 0.5 × SSC. The filter was kept at a particular temperature for 10 min, before the solvent was removed and its radioactivity measured. Radioactivity lost from the filter was recovered in solution, so that the radioactivity of the hybrids given in Table 1 also represents the radioactivity recovered in solution on heating from 25 to 99°C. The ordinate indicates the cumulative removal of radioactivity from the filter. The radioactive component was ^{125}I-labelled L-rRNA. (*a*) ○, *X. laevis* DNA·*X. laevis* L-rRNA after RNAase treatment; ●, *X. laevis* DNA·*N. crassa* L-rRNA after RNAase treatment; △, *X. laevis* DNA·*N. crassa* L-rRNA before RNAase treatment. (*b*): ○, *N. crassa* DNA·*N. crassa* L-rRNA after RNAase treatment; ●, *N. crassa* DNA·*X. laevis* L-rRNA after RNAase treatment; △, *N. crassa* DNA·*X. laevis* L-rRNA before RNAase treatment.

First, plasmid-pX1r101 DNA was restricted with endonucleases. The fragments were separated by agarose-gel electrophoresis. The DNA was then transferred to nitrocellulose film (Southern, 1975); those fragments carrying homologous sequences were then identified by hybridization with ^{125}I-labelled *N. crassa* L-rRNA. The principal result (see Fig. 2) is that homologies with the *N. crassa* probe are confined almost entirely to a *Hin*dIII/*Bam*HI fragment, extending over 3 kbp from the 3'-OH terminus of the L-rRNA gene.

Although 5.8S rRNA sequences account for approx. 3% of the *N. crassa* L-rRNA probe, no hybridization to the *X. laevis* 5.8S rRNA gene was detected. According to Boseley *et al.* (1978), this gene is located within fragment 4 (see Fig. 4), but no hybridization to this fragment produced by restriction endonucleases *Eco*RI and *Bam*HI was detected (see Fig. 3). In contrast, trace amounts (<5%) of S-rRNA present in the L-rRNA probe hybridized with the S-rRNA gene (Fig. 3). It is known that there are sequence homologies between 5.8S rRNA of different species; for example there are approx. 50% homologies between yeast and mammalian species (Erdmann, 1978, 1979). We infer that the duplex *X. laevis* 5.8S rDNA·*N. crassa* 5.8S rRNA is not stable under our conditions of hybridization, because the degree of mismatched residues is too high. It appears that there are fewer mismatched residues in *X. laevis* rDNA·*N. crassa* L-rRNA (or *X. laevis* rDNA·*N. crassa* S-rRNA) hybrids than in duplexes found between *X. laevis* rDNA and *N. crassa* 5.8S rRNA.

The distribution of approx. 1050 nucleotides of conserved sequences within the 3000-base-pairs fragment produced by restriction endonucleases *Hin*dIII and *Bam*HI was examined. ^{125}I-labelled *N. crassa* L-rRNA probe was found to hybridize with the rDNA fragment (see Fig. 4) produced by restriction with endonucleases *Bgl*II and *Hin*dIII (Fig. 2). Fragment 6 is located at the 5'-end of the 3000-base-pairs fragment (Fig. 4). The *N. crassa*

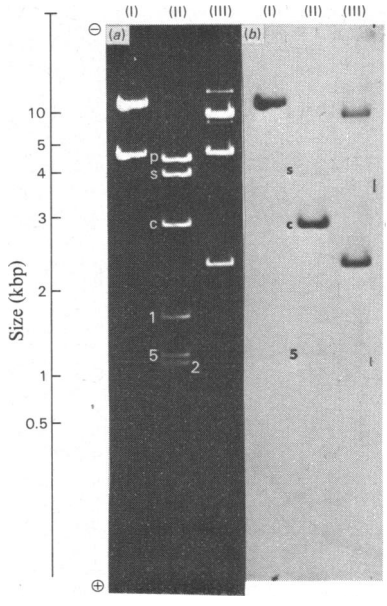

Fig. 2. *Restriction of X. laevis rDNA and analysis by agarose-gel electrophoresis and hybridization with N. crassa L-rRNA*

Plasmid-pX1r101 DNA was restricted with endonucleases. The fragments were separated by electrophoresis in 1% agarose gels. The DNA was transferred to nitrocellulose film (Southern, 1975) and hybridized with ^{125}I-labelled *N. crassa* L-rRNA at 65°C in 4 × SSC (see the Experimental section). (*a*) Agarose gel stained with ethidium bromide; (*b*) radioautograph of nitrocellulose film. Key: (I), restriction with endonuclease *Hin*dIII; (II), restriction with endonucleases *Hin*dIII/*Bam*I (double digestion); (III), restriction with endonucleases *Hin*dIII/*Bgl*II (double digestion); p, plasmid (pMB9) DNA; s, fragment that includes S-rRNA gene; c, conserved fragment of L-rRNA gene; 1, 2 and 5 are fragments defined in Fig. 4.

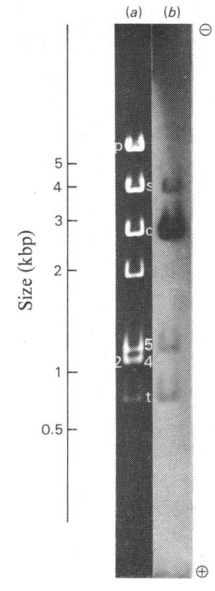

Fig. 3. *Restriction of X. laevis rDNA and hybridization of the 3′-OH-terminal fragment of the L-rRNA gene with N. crassa L-rRNA*

Plasmid-pX1r101 DNA was restricted with endonucleases *Eco*RI/*Bam*I (double digestion). The fragments were separated by electrophoresis in 1% agarose gels, transferred to nitrocellulose film and hybridized with ^{125}I-labelled *N. crassa* L-rRNA at 65°C in 4 × SSC. (*a*) Agarose gel stained with ethidium bromide; (*b*) radioautograph of nitrocellulose film. Key: p, plasmid DNA; s, fragment 3 carrying the S-rRNA gene [hybridization to s is accounted for by the presence of S-rRNA (< 5%) in the L-rRNA probe]; c, conserved fragment of L-rRNA gene; t, fragment having the 3′-OH end of L-rRNA; 2, 3, 4 and 5 are fragments defined in Fig. 4. Fragment 4 carries the 5.8 S-rRNA gene (Boseley *et al.*, 1978); it co-migrates with fragment 2, but does not hybridize with the 5.8 S-rRNA fraction of the *N. crassa* L-rRNA probe.

probe was also shown to hybridize with fragment 8 + 9 (Fig. 4) produced by restriction with endonucleases *Eco*RI and *Bam*HI. The 3′-OH terminus of the L-rRNA-coding region is contained in this *Eco*RI/*Bam*HI fragment. Fragments 6 and 8 + 9 are separated by 2100 base-pairs, so that the conserved sequences cannot form a single block.

An attempt was made to estimate the relative abundance of conserved sequences within a particular fragment by relating the relative blackening of the radioautograph with the size of the fragment, precautions being taken as outlined in the Experimental section. For example, after digestion with endonucleases *Bgl*II and *Bam*HI, 20% of the radioactivity (judged by subjecting the radioautograph to microdensitometry) was associated with fragment 6 (550 base-pairs), and 76% was identified with fragment 7 + 8 + 9, which comprises 2400 base-pairs of L-rRNA sequences. The ratio (number of conserved base-pairs/total number of base-pairs) was calculated (cf. Cox & Thompson, 1978) to be approx. 0.33 for the 3000-base-pairs fragment, for fragment 6 and for fragment 7 + 8 + 9.

The distribution of conserved sequences within the 3000-base-pairs fragment was further examined by restricting bacteriophage-λ RT9 DNA (as described in Fig. 5) with a variety of endonucleases. The capacity of the fragments produced to hybridize

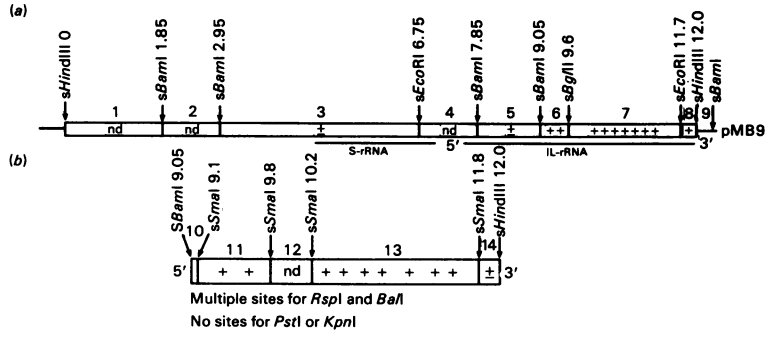

Fig. 4. *Summary of the hybridization of X. laevis rDNA with ^{125}I-labelled N. crassa L-rRNA*
(*a*) Summary of the data of Figs. 2 and 3. The data agree with a map made available by Dr. A. Bird and with the map by Boseley *et al.* (1978) for the s*Eco*RI 6750 base-pairs to s*Eco*RI 11750 base-pairs fragment. Hybridization to fragment 3 was due to traces of S-rRNA in the probe. The 5.8S-rRNA gene lies within fragment 4, but no hybridization to this fragment was detected. (*b*) Summary of the restriction data derived from digestion of the recombinant bacteriophage λ (see Figs. 5 and 6): ± indicates <5% of total radioactivity; + indicates the extent of hybridization indicated by the absorbance of the radioautograph; nd indicates that no hybridization was detected.

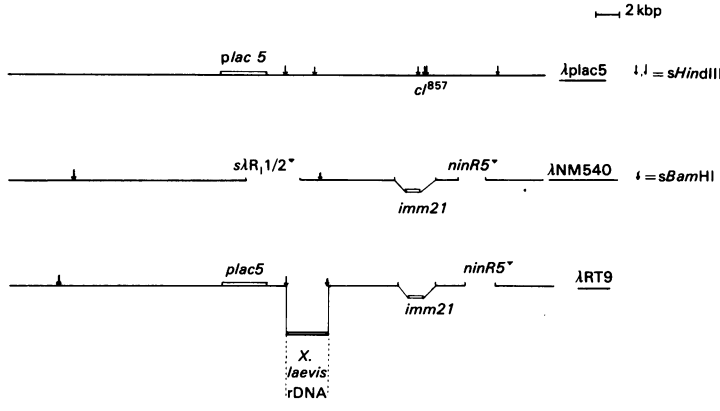

Fig. 5. *Construction of a recombinant formed from bacteriophage λ plac 5, bacteriophage λ NM540 and a fragment of X. laevis rDNA produced by restriction with endonucleases BamHI and HindIII*
The diagrams show the locations of the targets for restriction by endonucleases *Hin*dIII and *Bam*I, the *nin*R5 deletion and the immunity (*cI*^{857} or *imm*21) regions on the genomes of the bacteriophage. Key: ↓, endonuclease-*Hin*dIII sites; ↓, endonuclease-*Bam*HI sites. For simplicity *Hin*dIII sites alone are shown in bacteriophage λ p*lac*5 and *Bam*HI sites alone are shown in bacteriophage λ NM540. However, there is a *Bam*HI site at approx. 10% (15600 base pairs) from the left-hand end of bacteriophage λ p*lac*5, and this is shown in the diagram of recombinant bacteriophage λ RT9. Hence a double digest of recombinant yields two fragments from the bacteriophage-λ p*lac*5 sequences, the DNA insert(s), and the bacteriophage-λ NM540 fragment.

with nick-translated plasmid-pX1r101 DNA and with ^{125}I-labelled *N. crassa* L-rRNA was investigated.

Restriction with endonuclease *Sma*I yielded two fragments (0.7kbp and 1.6kbp) that hybridized with ^{125}I-labelled *N. crassa* L-rRNA. Using the notation of Fig. 4, specific sites for endonuclease *Sma*I are located at 9.1, 9.8 and 10.2kbp (cf. Boseley *et al.*, 1978). There must also be a site at 11.8kbp to account for the finding that the 1.6kbp fragment was found after restriction with endonuclease *Sma*I alone, as well as after digestion with endonucleases

*Hin*dIII/*Sma*I and *Sma*I/*Bam*I (Fig. 6). Conserved sequences were shown to be present in fragments 11 and 13 (Fig. 4*b*). Fragment 12 was found to hybridize with nick-translated plasmid-pX1r101 DNA, but did not appear to hybridize with the *N. crassa* probe, suggesting that within this fragment conserved sequences are either sparse or of low homology.

No site was detected within the *Bam*I/*Hin*dIII fragment for either of the endonucleases *Pst*I or *Kpn*I (Fig. 6). As expected (see Fig. 4) one *Eco*RI-specific site (s*Eco*RI 11.7 kbp) was identified. Restriction with endonuclease *Rsp*I yielded a fragment of 2.0 kbp that hybridized with ^{125}I-labelled *N. crassa* L-rRNA, whereas endonuclease *Bal*I yielded two fragments of 0.7 kbp and 1.2 kbp that hybridized with this probe. The sites for endonucleases *Rsp*I and *Bal*I were not identified. The results are summarized in Fig. 4(*b*).

Distribution of conserved sequences within the L-rRNA gene of N. crassa

Conserved sequences are not necessarily distributed in the same way in *N. crassa* as in *X. laevis*. For this reason *N. crassa* rDNA was investigated to establish whether the conserved sequences of L-rRNA form a single block or comprise multiple blocks alternating with non-conserved sequences.

A fragment of *N. crassa* rDNA of approx. 3400 base-pairs was produced by digestion with endo-

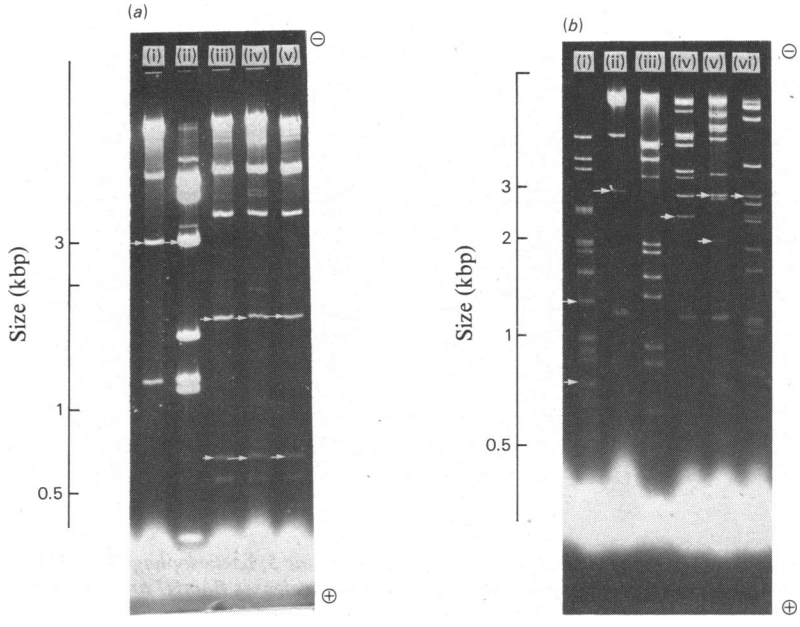

Fig. 6. *Restriction of X. laevis rDNA recombinant (bacteriophage λ RT9) with various enzymes and analysis by agarose-gel electrophoresis and hybridization with N. crassa L-rRNA*

DNA samples were restricted, fractionated on 1.5% (w/v) agarose gels, stained with ethidium bromide and transferred to nitrocellulose. Since each sample was run in duplicate, the nitrocellulose film was divided into two equivalent parts; one part was hybridized with ^{32}P-labelled nick-translated plasmid-pX1r101 DNA and the other with ^{125}I-labelled *N. crassa* L-rRNA at 65°C in 4 × SSC. Stained agarose gels are shown, and the arrows indicate fragments that hybridized with both ^{32}P-labelled plasmid-pX1r101 DNA probe and with *N. crassa* L-rRNA probe as shown by radioautography. (*a*) (i) Restriction of bacteriophage-λ RT9 DNA with endonucleases *Hin*dIII/*Bam*I (double digestion); (ii) restriction of plasmid-pX1r101 DNA with endonucleases *Hin*dIII/*Bam*I (double digestion); (iii) restriction of bacteriophage-λ RT9 DNA with endonucleases *Hin*dIII/*Sma*I (double digestion); (iv) restriction of bacteriophage-λ RT9 DNA with endonuclease *Sma*I; (v) restriction of bacteriophage-λ RT9 DNA with endonucleases *Bam*I/*Sma*I (double digestion). (*b*) (i) and (ii) Further restriction of bacteriophage-λ RT9 DNA with (i) endonucleases *Hin*dIII/*Bal*I (multiple digestion) or (ii) endonucleases *Hin*dIII/*Bam*I (double digestion); (iii) bacteriophage-λ $C_1$857 Sam7 DNA restricted with endonucleases *Hin*dIII/*Eco*RI (multiple digestion); (iv) bacteriophage-λ RT9 DNA restricted with endonucleases *Hin*dIII/*Bam*I/*Eco*RI (multiple digestion); (v) bacteriophage-λ RT9 DNA restricted with endonucleases *Hin*dIII/*Bam*I/*Rsp*I (multiple digestion); (vi) bacteriophage-λ RT9 DNA restricted with endonucleases *Hin*dIII/*Bam*I/*Pst*I (multiple digestion).

nuclease *Hin*dIII and was cloned in bacteriophage λ (Cox & Peden, 1979). This fragment includes all (or almost all) of the L-rRNA gene, including the 3′-OH terminus. Digestion with endonuclease *Eco*RI cleaves the rDNA fragment into two parts, one of 700 base-pairs and the other of 2700 base-pairs. The smaller fragment carries the 3′-OH terminus, although its exact location is uncertain. *N. crassa* L-rRNA was found to hybridize to the two fragments in the ratio 9 : 1, as judged by the intensity of radioautographs of the DNA fragment transferred to nitrocellulose film by the Southern (1975) procedure. This suggests that approx. 300 base-pairs of the smaller fragment code for L-rRNA. A similar result was obtained when ^{125}I-labelled *X. laevis* L-rRNA was used as probe (cf. Fig. 7*a*), i.e. both fragments were found to hybridize with probe in the

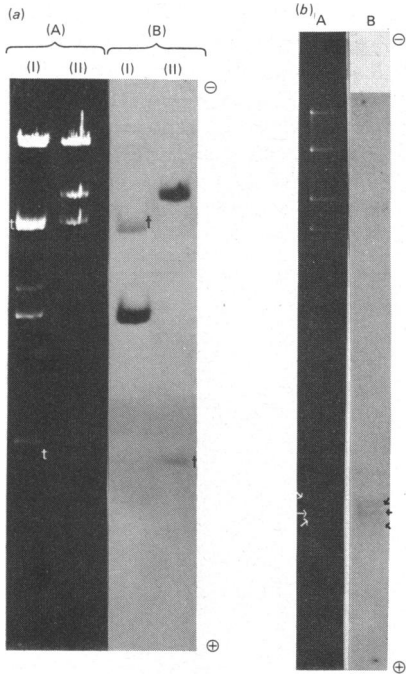

Fig. 7. *Restriction of N. crassa rDNA: analysis by agarose-gel electrophoresis and hybridization with ^{125}I-labelled X. laevis L-rRNA*

Bacteriophage λ N4 and N24 are molecular recombinants of bacteriophage λ 598 DNA and a 3400-base-pairs fragment of *N. crassa* rDNA that comprises most of the L-rRNA gene produced by restriction with endonuclease *Hin*dIII (Cox & Peden, 1979) that differ in the orientation of the inserted rDNA fragment. DNA from bacteriophages λ N4 and λ N24 was restricted with endonuclease *Eco*RI or *Sma*I, the fragments were separated by electrophoresis in 1% agarose, transferred to cellulose nitrate film by the Southern (1975) method and then hybridized with ^{125}I-labelled *N. crassa* L-rRNA at 65°C in 4 × SSC/0.1% SDS for 16h. (*a*) Restriction with endonuclease *Eco*RI: A, agarose gel stained with ethidium bromide; B, radioautograph of nitrocellulose film; (I), bacteriophage-λ N4 DNA; (II), bacteriophage λ N24 DNA; t indicates the 800-base-pairs fragment of *N. crassa* rDNA carrying the 3′-OH terminus of the L-rRNA-coding region. (*b*) Restriction of bacteriophage-λ N4 DNA with endonuclease *Sma*I. A, agarose gel stained with ethidium bromide; B, radioautograph of nitrocellulose film. The arrows indicate the bands of 800, 900 and 1000 base-pairs that are visible on the original negatives.

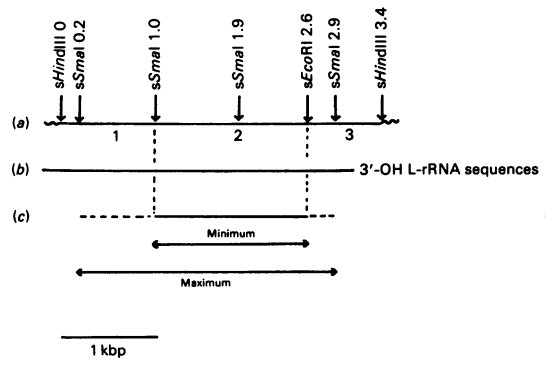

Fig. 8. *Distribution of conserved sequences within the N. crassa L-rRNA gene*

(*a*) Restriction map for bacteriophage-λ N24 DNA: ~ indicates bacteriophage-λ 598 sequences; s*Eco*RI etc. indicate the site for endonuclease *Eco*RI etc. measured in base-pairs from the *Hin*dIII site located close to the 5′-OH end of the L-rRNA gene. (*b*) Location of the L-rRNA gene (cf. Cox & Peden, 1979; Free *et al.*, 1979). ^{125}I-labelled *N. crassa* L-rRNA hybridized with the two *Eco*RI fragments in the ratio 8 : 1, suggesting that approx. 325 base-pairs of the 800-base-pairs fragment code for L-rRNA, and places the 3′-OH terminus at approx. 2925 base-pairs, i.e. close to the endonuclease-*Sma*I site at 2900 base-pairs. The site for endonuclease *Hin*dIII is approx. 200 base-pairs towards the 3′-OH end from the 5′-OH terminus. This terminus is located in an adjacent rDNA fragment (e.g. the 5200-base-pairs fragment produced by restriction with endonuclease *Hin*dIII (cf. Cox & Peden, 1979; Free *et al.*, 1979). The L-rRNA-coding region is estimated to be approx. 3200 base-pairs (approx. 1.21×10^6 daltons, based on a mean nucleotide mass of 379 for the K^+ salt). (*c*) The section of L-rRNA spanning the conserved sequences. This section must extend towards the 5′-OH end from the endonuclease-*Sma*I site at 1000 base-pairs and towards the 3′-OH end from the endonuclease-*Eco*RI site at 2600 base-pairs (see Fig. 7). Thus the conserved sequences must span at least 1600 base-pairs and not more than 2700 base-pairs.

ratio of 9:1 judged by the intensity of the radioautograph. The endonuclease *Sma*I cleaved the rDNA fragment into three major pieces of approx. 800, 900 and 1000 base-pairs. Each of these fragments was found to hybridize with the *X. laevis* L-rRNA probe (see Fig. 7*b*). On the basis of the known position of the sites for endonucleases *Eco*RI and *Sma*I (Cox & Peden, 1979), the 1050 base-pairs that comprise the conserved sequences must be distributed over a span of not less than 1600 base-pairs and not more than 2700 base-pairs (see Fig. 8). We conclude that the conserved sequences form more than one block.

Distribution of sequences common to X. laevis and D. melanogaster L-rRNA

It is likely that sequences common to *X. laevis* and *N. crassa* L-rRNA represent an element of all cytoplasmic ribosomes if these elements are essential to the ribosome. According to this view, L-rRNA sequences of another species should also hybridize with the sequences shared by *X. laevis* and *N. crassa*; *D. melanogaster* rDNA was chosen as another probe to test this notion. This choice was influenced by the knowledge that *Xenopus* (67% G+C) and *Drosophila* (42% G+C) represent the extremes of nucleotide composition of L-rRNA (Lava-Sanchez *et al.*, 1972). Allowing for a core of conserved sequences, the non-conserved parts of the two

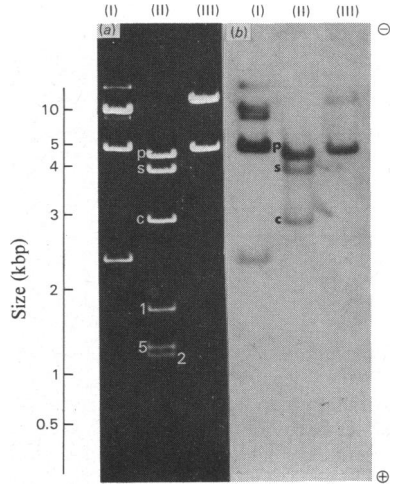

Fig. 9. *Restriction of X. laevis rDNA, analysis by agarose-gel electrophoresis and hybridization with* ^{32}*P-labelled D. melanogaster rDNA*

Plasmid-pX1r101 DNA was restricted with endonucleases. The fragments were separated by electrophoresis in 1% agarose gels. The DNA was transferred to nitrocellulose film (Southern, 1975) and hybridized with ^{32}P-labelled nick-translated plasmid-pDM103 DNA at 65°C in 4 × SSC/0.2% SDS for 16 h. (*a*) Agarose gel stained with ethidium bromide; (*b*) radioautograph of cellulose film. (I) Restriction with endonucleases *Hin*dIII/*Bgl*II (double digestion); (II) restriction with endonucleases *Hin*dIII/*Bam*HI (double digestion); (III) restriction with endonuclease *Hin*dIII. Key: p, plasmid (pMB9) DNA; s, DNA fragment that includes the S-rRNA gene; c, conserved fragment of L-rRNA gene; 1, 2 and 5 are fragments defined in Fig. 4. The *D. melanogaster* DNA probe contains both the S-rRNA and L-rRNA genes. The probe also hybridized with plasmid-pMB9 sequences, because plasmid pMB9 is a derivative of plasmid pSC101, which is the vector moiety of plasmid pDM103 (Glover *et al.*, 1975).

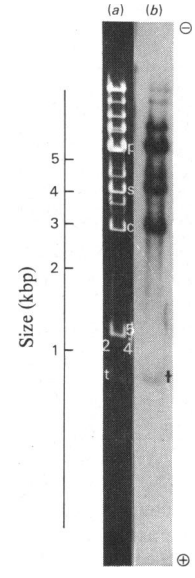

Fig. 10. *Restriction of X. laevis rDNA and hybridization of the 3'-OH-terminal fragment of the L-rRNA gene with* ^{32}*P-labelled D. melanogaster (plasmid pDM103) rDNA*

Plasmid p-4305 DNA (a molecular recombinant of pCM2 DNA and *X. laevis* rDNA) was restricted with endonucleases *Eco*RI/*Bam*HI (double digestion). The fragments were separated by electrophoresis in 1% agarose gels transferred to nitrocellulose film and hybridized with ^{32}P-labelled (nick-translated) plasmid-pDM103 DNA at 65°C in 4 × SSC/0.2% SDS fopr 16 h. (*a*) Agarose gel stained with ethidium bromide; (*b*) radioautograph of nitrocellulose film. Key: p, plasmid DNA; s, DNA fragment including the S-rRNA gene; t, fragment having the 3'-OH terminus of L-rRNA. Products of partial disgestion are also present; c, conserved fragment [6 + 7 (see Fig. 4)]. The probe also hybridized with plasmid-pCM2 sequences, because this is a derivative of plasmid pSC101, which is the vector moiety of plasmid pDM103 (Glover *et al.*, 1975).

species must be even more extreme in their base composition. Investigation supports this view, since substantial tracts of approx. 76% G + C were found in *X. laevis* (Cox *et al.*, 1973), and tracts of approx. 25% G + C were identified in *D. melanogaster* L-rRNA (Cox *et al.*, 1976). Nick-translated plasmid-pDM103 DNA was used as the probe for *D. melanogaster*. The cloned sequences include both S- and L-rRNA genes (Glover *et al.*, 1975). After restriction of plasmid-pX1r101 DNA, the fragments were separated by electrophoresis in 1% agarose gels, transferred to nitrocellulose filters and hybridized with the *Drosophila* probe. After restriction with endonucleases *Bam*HI and *Hin*dIII, or with endonucleases *Bgl*II and *Hin*dIII, it was found that the same fragments of the L-rRNA gene that hybridized with the *N. crassa* probe (Fig. 2) also hybridized with the *Drosophila* probe (Fig. 9). The *X. laevis* rDNA carrying the 3′-OH terminus of the L-rRNA gene, produced by restriction with endonucleases *Bam*HI and *Eco*RI, also hybridized with the *Drosophila* probe (see Fig. 10; cf. Fig. 3). These data do not prove that the same sequences of *X. laevis* are involved in hybridization with *D. melanogaster* as in hybridization with *N. crassa*, although the evidence is consistent with this notion. Gerbi (1976) studied the hybridization of *Drosophila hydei* DNA with *X. laevis* S- plus L-rRNA and found that 20% of rRNA sequences were in common, with less than 10% of mismatched residues within the conserved sequences. Gerbi (1976) also showed that mouse rRNA competed with *X. laevis* rRNA for *D. hydei* rDNA in hybridization experiments. This result favours the notion that sequences common to *X. laevis*, mouse and *D. hydei* rDNA are also present in *N. crassa*.

Distribution of sequences common to N. crassa and D. melanogaster

Nick-translated *D. melanogaster* rDNA hybridized with the same fragments of *N. crassa* rDNA produced by restriction of bacteriophage-λ N4 DNA (or bacteriophage-λ N24 DNA) with endonucleases *Eco*RI and *Sma*I as did ^{125}I-labelled *X. laevis* L-rRNA (cf. Figs. 7 and 11). According to the restriction map of bacteriophage-λ N24 (see Fig. 8), sequences common to *N. crassa* and *D. melanogaster* must be spread over a range of 1600–2700 base-pairs of the *N. crassa* L-rRNA gene.

The converse experiment, hybridization of *N. crassa* L-rRNA with *D. melanogaster* rDNA, was also performed. The L-rRNA-coding region of *D. melanogaster* rDNA was analysed further by making use of the plasmids cKDm103A etc. constructed by Glover & Hogness (1977) by cloning fragments of plasmid pDM103, produced by endonuclease *Hin*dIII, in plasmid pML2. The L-rRNA-coding region is split into three pieces by endonuclease *Hin*dIII, i.e. into fragments of 3300 base-pairs, 200 base-pairs and 700 base-pairs, reading from 5′-end to 3′-OH end. These fragments were cloned and yielded respectively plasmids cKDm103A, cKDm103B, and cKDm103C. Two of the plasmids were studied, namely cKDm103A and

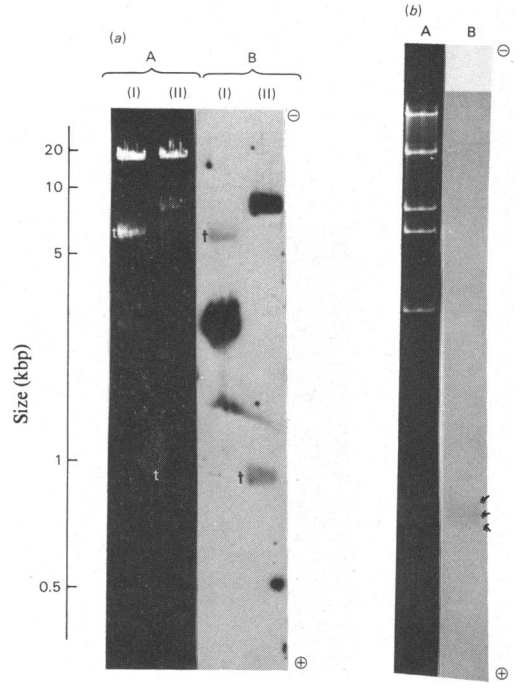

Fig. 11. *Restriction of N. crassa rDNA, analysis by agarose-gel electrophoresis and hybridization with* ^{32}P-*labelled nick-translated D. melanogaster rDNA*

The bacteriophage-λ 598–*N. crassa* rDNA molecular recombinants λ N4 and λ N24 (for details see the legend to Fig. 7) were restricted with endonucleases *Eco*RI or *Sma*I, the fragments were separated by electrophoresis in 1% agarose, transferred to nitrocellulose film by the method of Southern (1975) and then hybridized with ^{32}P-labelled *D. melanogaster* rDNA (plasmid-pDM103 DNA) at 65°C in 4 × SSC/0.1% SDS for 16 h. (*a*) Restriction with endonuclease *Eco*RI: A, agarose gel stained with ethidium bromide; B, radioautograph of nitrocellulose film; (I), λ-N4 DNA; (II), λ-N24 DNA; t indicates the 700-base-pairs fragment of *N. crassa* rDNA carrying the 3′-OH terminus of the L-rRNA-coding region. (*b*) Restriction of bacteriophage-λ N24 DNA with endonuclease *Sma*I. A, agarose gel stained with ethidium bromide; B, radioautograph of the nitrocellulose film. The arrows indicate the bands of 800, 900 and 1000 base-pairs that are visible on the original negatives.

cKDm103C, and both hybridized with ^{125}I-labelled *N. crassa* L-rRNA probe (see Fig. 12).

Hybridization of *N. crassa* probe with plasmid-cKDm103C DNA shows that sequences within 700 base-pairs of the 3′-OH terminus of *D. melanogaster* L-rRNA gene are common to both species. Conserved sequences were detected within 300 base-pairs of the 3′-OH terminus of the *X. laevis* L-rRNA gene (see Figs. 3 and 9), within approx. 600 base-pairs of the *N. crassa* L-rRNA gene (see Figs. 7 and 11), as well as within 700 base-pairs of the *D. melanogaster* L-rRNA gene. Hence it is likely that conserved sequences within 300 base-pairs of the *X. laevis* L-rRNA gene hybridize with sequences within 600 base-pairs of the 3′-OH end of *N. crassa* L-rRNA and with sequences within 700 base-pairs of *D. melanogaster* L-rRNA.

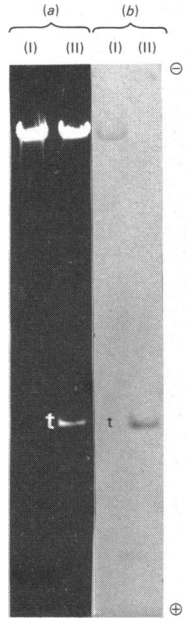

Fig. 12. *Hybridization of D. melanogaster rDNA fragments with ^{125}I-labelled N. crassa L-rRNA*
DNA from plasmids pCK103A and pCK103C was restricted with endonuclease *Hin*dIII to liberate the cloned *D. melanogaster* rDNA fragments. The fragments were separated on 1% agarose gels, transferred to nitrocellulose film and hybridized at 65°C with 4 × SSC/0.1% SDS for 16h. (*a*) Agarose gels stained with ethidium bromide; (*b*) radioautograph of nitrocellulose film. (I) plasmid-pCK103 DNA; the vector DNA (plasmid-pML2 DNA, 13700 base-pairs) and inserted DNA (9840 base-pairs, containing a fragment of 3300 base-pairs of the L-rRNA gene, including the 5′-OH terminus) are not separated by the agarose gel. (II) plasmid-pCK103C DNA; t indicates the 700-base-pairs *D. melanogaster* rDNA insert that contains the 3′-OH terminus of the L-rRNA gene (Glover & Hogness, 1977).

Discussion

The presence of nucleotide sequences common to both *X. laevis* and *N. crassa* rRNA was first shown by the isolation of hybrids formed between *X. laevis* rDNA and *N. crassa* rRNA (Sinclair & Brown, 1971). This study shows that the conserved regions of L-rRNA comprise approx. 1050 nucleotides, and that the fidelity of base-pairing between rDNA of one species and L-rRNA of the other is 90% or better. Both the extent of homology and the high degree of matching between homologous L-rRNA sequences confirm that particular sequences have been highly conserved during evolution (cf. Sinclair & Brown, 1971).

We have shown that in both *X. laevis* and *N. crassa* rDNA regions of high and low homology must be intermingled over a span of up to 3000 bases. The sizes of the blocks have yet to be established. Although in both species (and also in *D. melanogaster*) conserved sequences were shown to be present within several hundred base-pairs of the 3′-OH end of the L-rRNA gene, it remains to be shown whether, reading from the 5′-end, conserved sequences occur in the same order in both *X. laevis* and *N. crassa* L-rRNA. Both questions may be resolved when nucleotide-sequence data are available.

The paucity of homologies at the 5′-end of the *X. laevis* L-rRNA gene correlates (see Fig. 13) with the presence of bihelical structures at the 5′-end of L-rRNA that survive the conditions used for spreading samples for electron microscopy (Wellauer & Dawid, 1974, 1976; for review see Wellauer & Dawid, 1977). These structured regions also correspond to bihelical parts rich in G and C residues (approx. 76% G + C), accounting for approx. 1500 bases, that were isolated as large fragments from partial nuclease digests (Cox *et al.*, 1973; Godwin *et al.*, 1974). Thus it appears that approx. 1000 residues at the 5′-end of *X. laevis* L-rRNA are not only G + C-rich (approx. 76% G + C) and form relatively stable bihelical structures, but also they have few, if any, homologies with *N. crassa* L-rRNA or *D. melanogaster* L-rRNA. It was suggested previously (Cox *et al.*, 1973) that the tracts rich in G and C residues are mainly non-conserved sequences. Spectrophotometric measurements were also interpreted to provide a rough estimate of the base composition of conserved parts of *X. laevis* L-rRNA, i.e. 50–55% G + C (Cox *et al.*, 1973).

Table 2. *Summary of properties of L-rRNA species examined*

References: [1]data from Loening (1968); [2]data from Lava-Sanchez *et al.* (1972); [3]Cox *et al.* (1973); [4]Cox *et al.* (1976).

L-rRNA	$10^{-6} \times$ Mass[1] (daltons)	Nucleotide composition[2] (mol %) AMP	CMP	GMP	UMP	G + C content (%)	$10^{-6} \times$ Mass (daltons) of conserved sequences	G + C content of non-conserved sequences (%)
X. laevis	1.3	17	30	37	16	67	0.38 ± 0.06	78[3]
N. crassa	1.5	24.8	21.9	29.4	23.9	51.3	0.38 ± 0.06	—
D. melanogaster	1.4	30.8	19.6	22.5	27.1	42.1	0.28*	25[4]

* Estimated from the data of Gerbi (1976) for the hybrid formed between *D. hydei* DNA and *X. laevis* rRNA, and included on the basis of the assumption that the rRNA genes of the two species of *Drosophila* have very similar properties.

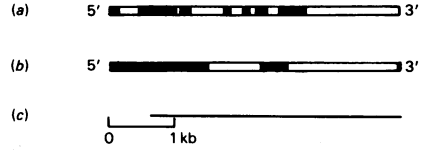

Fig. 13. *Comparison of the distribution within the L-rRNA gene of X. laevis of non-conserved sequences with the distribution within L-rRNA of regions of secondary structure of high thermal stability*

(*a*) Secondary-structure map of *X. laevis* L-rRNA (Wellauer & Dawid, 1974): ■ denotes region of secondary structure detected by electron microscopy after spreading L-rRNA by means of a denaturing solvent. The length indicated corresponds to the estimated single-strand length of the hairpin loop. These regions of stable secondary structure are identified as (G + C)-rich on the basis of spectrophotometric analysis and partial nuclease digests (Cox *et al.*, 1973) and also electron microscopy (Godwin *et al.*, 1974). □, Regions appearing as a single-strand. (*b*) Distribution of non-conserved sequences identified in this study (see Fig. 4): ■ indicates regions of very low homology with heterologous probes. (*c*) Size of *N. crassa* L-rRNA gene (Cox & Peden, 1979).

Conserved sequences and ribosome function

We infer that sequences common to *X. laevis* and *N. crassa* L-rRNA are important because they are essential to the ribosome for either assembly, or function, or both. Implicit in this inference is the assumption that the sequences common to these two species represent a general feature of cytoplasmic L-rRNA. There is some direct evidence to support this assumption (Gerbi, 1976). The indirect evidence includes studies that show that *X. laevis* rRNA shares sequences in common with a very wide range of species irrespective of their overall nucleotide composition (Sinclair & Brown, 1971; Birnstiel & Grunstein, 1971; cf. Martin *et al.*, 1970). We have shown above that *X. laevis* rDNA and *N. crassa* rDNA both hybridize with *D. melanogaster* rDNA, even though the nucleotide composition of the L-rRNA genes span the range 42–67% G + C.

To reconcile the evidence that L-rRNA has diverged during evolution with the evidence for conserved features, it was suggested (Cox *et al.*, 1976) that the molecule comprises a core of highly conserved sequences combined with non-conserved sequences whose abundance and nucleotide composition varies from class to class (see also Table 2). On the basis of this hypothesis, the sequences common to *N. crassa* and *X. laevis* comprise part of the conserved core. This hypothesis does not preclude the possibility that in certain cases, e.g. *X. laevis* and mammalian L-rRNA, which are both G + C-rich, sequence homologies may exist in the so-called non-conserved regions, in addition to homologies arising from the conserved core.

It is possible that L-rRNA may have a direct role in the peptidyltransferase function because parts are sufficiently close to the active site of the ribosome to combine with reactive substrate analogues (Greenwell *et al.*, 1974; Breitmeyer & Noller, 1976). Also, the tertiary structure of L-rRNA, as maintained by Mg^{2+}, appears to be important to the peptidyltransferase centre of the ribosome (Cox & Greenwell, 1976). If L-rRNA does form part of the active site of the ribosome, it is probable that 'conserved' sequences are involved. Many features of the active centre of the ribosome must be common to all species, because they all share the same substrate, namely mRNA, aminoacyl-tRNA and peptidyl-tRNA. In contrast, the significance of the non-conserved parts of L-rRNA is not apparent, and there are few indications of their function.

Finally, a knowledge of the nucleotide sequence of the conserved parts of L-rRNA is needed to establish the extent of homologies between different

species and so further our understanding of the structure and function of the ribosome.

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References

Allet, B. & Bukhari, A. (1975) *J. Mol. Biol.* **92**, 529–540
Birnstiel, M. L. & Grunstein, M. (1972) *FEBS Symp.* **23**, 349–364
Bonner, T. I., Brenner, O. J., Neufeld, B. R. & Britten, R. J. (1973) *J. Mol. Biol.* **81**, 123–135
Boseley, P. G., Tuyns, A. & Birnstiel, M. L. (1978) *Nucleic Acids Res.* **5**, 1121–1137
Breitmeyer, J. B. & Noller, H. F. (1976) *J. Mol. Biol.* **101**, 297–306
Clewell, D. D. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **62**, 1159–1166
Cox, R. A. (1966) *Biochem. Prep.* **11**, 104–109
Cox, R. A. (1977) *Prog. Biophys. Mol. Biol.* **32**, 193–231
Cox, R. A. & Greenwell, P. (1976) *Biochem. J.* **160**, 521–531
Cox, R. A. & Hirst, W. (1976) *Biochem. J.* **160**, 505–519
Cox, R. A. & Peden, K. (1978) *Biochem. Soc. Trans.* **6**, 1230–1232
Cox, R. A. & Peden, K. (1979) *Mol. Gen. Genet.* **174**, 17–24
Cox, R. A. & Thompson, R. (1978) *Biochem. Soc. Trans.* **6**, 1232–1233
Cox, R. A., Godwin, E. A. & Huvos, P. (1973) *Isr. J. Chem.* **11**, 407–422
Cox, R. A., Godwin, E. A. & Hastings, J. R. B. (1976) *Biochem. J.* **155**, 465–475
Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646
Erdmann, V. A. (1978) *Nucleic Acids Res.* **5**, r1–r13
Erdmann, V. A. (1979) *Nucleic Acids Res.* **6**, r29–r44
Free, S. J., Rice, P. W. & Merzenberg, L. (1979) *J. Bacteriol.* **137**, 1219–1226
Gerbi, S. (1976) *J. Mol. Biol.* **106**, 791–816
Gillespie, D. & Spiegelman, S. (1965) *J. Mol. Biol.* **12**, 829–842
Glover, D. M. & Hogness, D. S. (1977) *Cell* **10**, 167–176
Glover, D. M., White, R. L., Finnegan, D. J. & Hogness, D. S. (1975) *Cell* **5**, 149–157
Godwin, E. A., Cox, R. A. & Huvos, P. (1974) *Acta Biol. Med. Ger.* **33**, 733–752
Greenwell, P., Harris, R. J. & Symons, R. H. (1974) *Eur. J. Biochem.* **49**, 4539–4554
Horwitz, J. P., Chua, J., Curby, R. J., Tomson, A. J., Da Rooge, M. A., Fisher, B. E., Mauricio, J. & Klundt, I. (1964) *J. Med. Chem.* **7**, 574–583
Laskey, R. A. & Mills, A. D. (1977) *FEBS Lett.* **82**, 314–316
Lava-Sanchez, P. A., Amaldi, F. & La Posta, A. (1972) *J. Mol. Evol.* **2**, 44–45
Loening, U. E. (1968) *J. Mol. Biol.* **38**, 355–365
Maniatis, T., Jeffreys, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1184–1188
Martin, T. E., Bicknell, J. N. & Kumar, A. (1970) *Biochem. Genet.* **4**, 603–615
Murray, K. & Murray, N. E. (1975) *J. Mol. Biol.* **98**, 551–564
Murray, N. E. & Murray, K. (1974) *Nature (London)* **251**, 476–481
Murray, N. E., Brammar, W. J. & Murray, K. (1977) *Mol. Gen. Genet.* **150**, 53–61
Pratt, H. & Cox, R. A. (1971) *Biochem. J.* **124**, 897–903
Sinclair, J. H. & Brown, D. D. (1971) *Biochemistry* **10**, 2761–2769
Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517
Thomas, C. A. & Dancis, B. M. (1973) *J. Mol. Biol.* **77**, 43–55
Wellauer, P. K. & Dawid, I. B. (1974) *J. Mol. Biol.* **89**, 379–395
Wellauer, P. K. & Dawid, I. B. (1976) *Cell* **8**, 443–448
Wellauer, P. K. & Dawid, I. (1977). in *Recombinant Molecules: Impact on Science and Society* (Beers, R. F. & Bassett, E. G., eds.), pp. 379–398, Raven Press, New York